INVALID

MRID No. 416060-03

DATA EVALUATION RECORD

1. **CHEMICAL:** Bromoxynil octanoate
Shaughnessey No. 035301 and 035302

2. **TEST MATERIAL:** Bromoxynil octanoate technical (2,6-dibromo-4-cyanophenyl octanoate), M & B Lot No. CN-51033 (20-DLM-152-1), 97.2% active ingredient, a brown solid.

3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants -- Tier 2. Species Tested: Lemna gibba G3.

4. **CITATION:** Giddings, J.M. 1990. Bromoxynil octanoate-Toxicity to the Duckweed Lemna gibba G3. SLI Report No. 90-8-3430. SLI Study No. 10566.1089.6144.410. Conducted by Springborn Laboratories, Inc., Wareham, Massachusetts. Submitted by Rhone-Poulenc Ag Company, Research Triangle Park, NC. EPA MRID No. 416060-03.

5. **REVIEWED BY:**

Marise Robbins, M.A., M.S.E.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: M. Robbins
Date: 2/10/91 2/12/91

6. **APPROVED BY:**
Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 2/12/91

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature: Henry T. Craven
Date: 2/19/91

7. **CONCLUSIONS:** This study is not scientifically sound and does not fulfill the guideline requirements for growth and reproduction of aquatic plants (Tier 2) for the following reasons: 1) The actual exposure concentrations are not known, and 2) The test temperature fluctuated from 19 to 29°C.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

7 hrs

11. **MATERIALS AND METHODS:**

A. **Test Species:** Lemna gibba used in this test came from laboratory stock cultures. The original cultures were obtained from C.F. Cleland, USDA, Washington, D.C. The stock cultures were maintained in M-type Hoagland's medium (without EDTA) which was prepared with deionized water and adjusted to pH 5.0 with 0.1N sodium hydroxide after autoclaving.

Stock cultures were grown in sterile 270-ml covered crystallizing dishes containing 100 ml of medium. Stock culture transfers were made approximately once or twice per week into fresh media. The inoculum used to initiate the toxicity test with Bromoxynil was taken from a stock culture that had been transferred to fresh medium 3 days before testing.

The stock cultures were maintained under test conditions (temperature of 25 ± 2°C, continuous illumination at the surface of the medium of approximately 300-400 footcandles) for 3 days before testing. Temperature was controlled using an environmental chamber. Lighting was supplied by Vita-Light® fluorescent lights.

B. **Test System:** All test vessels and control vessels were 270-ml crystallizing dishes conditioned with the appropriate test solution and filled with 100 ml of medium. Each dish was covered with an inverted, sterile, glass petri dish. The M-type Hoagland's medium was prepared in approximately the same way as the culture medium. The test medium was prepared with deionized water, autoclaved and brought to room temperature. The pH of this medium was adjusted to 5.0 with 0.1N potassium hydroxide, rather than the sodium hydroxide used for the culture medium.

The environmental conditions during the test were approximately the same as during culturing, with a greater variance in temperature and light intensity. The temperature was maintained at 19-29°C. Fluorescent lighting was continuous at an intensity of 170-525 footcandles at the solution surface.

The primary stock solution of 8.0 mg a.i./L was prepared by adding 0.8232 g of Bromoxynil (0.8002 g as a.i.) to a sterile 100-ml volumetric flask and diluting to volume with acetone. The 0.8-mg a.i./L nominal test solution was prepared by diluting a subsample of the

primary stock with the medium. The remaining nominal test concentrations were prepared in 500-ml volumetric flasks by diluting appropriate volumes of the primary stock with acetone to create the secondary stock and then diluting equal volumes (0.05 ml) of each secondary stock with the medium. One hundred ml of the appropriate test solution were then placed in each 270-ml conditioned crystallizing dish.

The control contained test medium with no additions. The solvent control contained acetone diluted with medium and was equal to the amount of solvent present in each of the test solutions (i.e., 0.1 ml/L).

C. **Dosage:** Fifteen-day growth and reproduction test (3-day renewal intervals). Nominal and measured test concentrations were reported as mg Bromoxynil (as active ingredient)/L solution. Based on a range-finding test, five nominal concentrations of 0.80, 0.40, 0.20, 0.10, and 0.052 mg a.i./L were selected for the definitive test.

D. **Design:** The test was initiated 30 minutes after the test solutions were added to the test dishes. Three replicates of each treatment level and control were used in the test. Five plants, consisting of three fronds each for a total of fifteen fronds (from 3-day-old stock cultures), were aseptically added to each test vessel.

The flasks were kept in an environmental chamber. Temperature was measured continuously with a Taylor minimum/maximum thermometer. Light intensity of the test area was measured daily with a General Electric type 214 light meter. The pH of each treatment level was measured with a LaMotte model HA pH meter at test initiation, in the old and new solutions at each 3-day interval, and at test termination. At test termination, replicate solutions from the test containers were composited for 15-day pH measurements.

At each 3-day interval (3, 6, 9, 12 and 15 days after initiation), fronds were counted and plants transferred aseptically into sterile crystallizing dishes containing fresh test solutions. Flasks were randomly repositioned every 3 days, after transfers to fresh medium, to minimize spatial differences in the environmental chamber.

Observations consisted of recording frond production

3

and appearance over time. As fronds age and die, they lose their pigmentation and become chlorotic. The number of chlorotic (yellow) fronds was indicated and included in the total frond count.

All solutions were analyzed for Bromoxynil (as the phenolic degradate) by high pressure liquid chromatography.

Additionally, 3 quality control (QC) samples were prepared at each interval using fresh medium at concentrations similar to the treatment level range. The QC samples remained with the set of test samples through analysis. The QC results were used to judge the quality of the analytical process.

E. **Statistics:** A t-test was used to compare frond counts in controls with frond counts in solvent controls. If controls and solvent controls were not significantly different ($p = 0.01$), data from the two sets of controls were pooled for further analysis. If significant differences were found between controls and solvent controls, data from the solvent control were used for statistical comparison.

Because the Bromoxynil concentrations did not result in a 50% reduction in frond production as compared to the pooled controls, EC values were not calculated.

The no-observed-effect-concentration (NOEC) was determined using one-way analysis of variance (ANOVA) and Dunnett's Procedure if all treatment groups had the same number of replicates (i.e., control data were not pooled), or Bonferroni's Test if the treatment groups had unequal numbers of replicates (i.e., control data were pooled). Before conducting the ANOVA, the data were checked for normality and homogeneity of variance.

12. **REPORTED RESULTS:** The results of the analysis of test solutions for Bromoxynil are summarized in Table 2 (attached).

Bromoxynil concentrations were measured in the fresh test solutions at Day 12 and in the old test solutions on Day 15. In the fresh test solutions with nominal concentrations of 0.8-0.1 mg a.i./L, measured concentrations ranged from 32 to 111% of nominal. The lowest nominal treatment level resulted in a measured concentration of <0.025 mg a.i./L (the minimum detectable level). All exposure solutions were below

the minimum detectable level on Day 15. The treatment levels were defined (based on the results of the day-12 analysis) as 0.25, 0.18, 0.22, 0.076, and <0.025 mg a.i./L. The three Quality Control samples averaged 77% and 103% recovery respectively, on days 12 and 15.

Table 3 (attached) presents frond production and observations recorded during the 15-day test for each mean measured concentration. Statistical analysis demonstrated that frond production in solvent control cultures was not significantly different from that of the control cultures after 15 days. Therefore, the data from both solvent control and control were pooled.

At test termination, frond production was reduced by 29.3% in the highest concentration tested and was significantly different ($p = 0.05$) from the pooled control. Table 4 (attached) indicates the percent reduction in the remaining concentrations tested. In general, fronds exposed to Bromoxynil concentrations ranging from 0.25 to 0.076 mg a.i./L remained healthy.

Statistical analysis used Bonferroni's Test since controls had been pooled. This analysis established an NOEC of 0.22 mg a.i./L. Temperature and pH measurements are presented in Table 5 (attached). The pH of the test solutions ranged from 4.9 to 6.2 throughout the test. Light intensity ranged from 170 to 525 footcandles. Temperature ranged from 19-29°C throughout the study period.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** Since no concentration of Bromoxynil tested resulted in ≥ 50% reduction no EC values were calculated. The NOEC established for this study was 0.22 mg a.i./L. The highest concentration tested, 0.25 mg a.i./L, caused less than 50% reduction in frond production. The maximum concentration of Bromoxynil that would occur if the compound were applied to a 15-cm water column is 0.275 mg a.i./L.

Several inspections had been conducted during the course of the study by the Springborn Laboratories, Inc. Environmental Sciences Division Quality Assurance Unit to assure adherence to the study protocol, laboratory standard operating procedures and the pertinent EPA Good Laboratory Practice Regulations.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedure and the report were

5

generally in accordance with the SEP and Subdivision J guidelines, except for the following deviations:

o This study was a fifteen day test instead of 14 days as recommended in the guidelines. This change in protocol was reported by the laboratory and allowed for a 3-day renewal period between days 12 and 15.

o The SEP states that the temperature should be maintained at 25 ± 2°C. In this study, the temperature ranged from 19-29°C.

o The SEP states that the light intensity should be maintained at approximately 5000 lux (app. 465 footcandles). In this study, the light intensity during the test ranged from 170-525 footcandles at the solution surface.

B. **Statistical Analysis:** Analysis of variance with multiple comparison tests was performed to compare frond production at each treatment level to those of the controls. The controls were not pooled in this analysis. Newman-Keuls' test and Tukey-A test showed that no test concentrations significantly ($p \leq 0.05$) reduced frond production of L. gibba. This statistical analysis established an NOEC for the study as 0.25 mg a.i./L, which is different from the author's. The difference is due to the fact that the author compared the frond production at each treatment level to that of the pooled control (higher yield). Since the author's result is more conservative, 0.22 mg a.i./L is accepted as the NOEC for the test.

Since all tested concentration levels caused a reduction of < 50% frond production, no EC50 was calculated. Therefore, the EC50 is considered >0.25 mg a.i./L.

C. **Discussion/Results:** This study is not scientifically sound. Chemical analysis of the test solutions during the last renewal period (day 12 and day 15) shows inconsistencies in measured concentrations (Table 2, attached). The measured concentrations ranged from 32 to 111% of the nominals in fresh solutions (day 12) and all test levels were below the detection limit (0.025 mg a.i./L) by day 15.

In addition, an accompanying report for Navicula pelliculosa tested with the same test material (MRID #416060-01) indicated that there was a problem with the

6

test material solubility in water. Since the samples of test solutions collected were not filtered before the chemical analysis, the actual exposure concentrations in this test are not known.

There was a rather large variance in Bromoxynil recovery rates among the three Quality Control samples on days 12 and 15, which averaged 77% and 103%, respectively. This variance is greater than that expected to result from equipment and operator error.

The temperature during testing had a range (19-29°C) greater than required by protocol (25 ± 2°C). This could have affected the metabolism of L. gibba.

D. **Adequacy of the Study:**

(1) **Classification:** Invalid.

(2) **Rationale:** 1) Actual exposure concentrations are not known, 2) test temperature fluctuated from 19 to 29°C.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER:** Yes, January 30, 1991.

7

Analysis of Variance   File: bromlem   Date: 01-22-1991

FILTER: None

N's, means and standard deviations based on dependent variable: FROND

* Indicates statistics are collapsed over this factor

Factors: C	Conc. (mg Al/L)	N	Mean	S.D.
*		21	516.5714	88.4356
1	Control	3	520.3333	53.5381
2	Solvent control	3	558.0000	52.8867
3	<0.025	3	534.6667	24.1730
4	0.076	3	545.0000	58.1034
5	0.22	3	601.6667	40.7717
6	0.18	3	475.6667	38.6825
7	0.25	3	380.6667	141.5992

DDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDD

Fmax for testing homogeneity of between subjects variances: 34.31
Number of variances= 7   df per variance= 2.

DDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDD

Analysis of Variance   Dependent variable: FROND

Source	df	SS (H)	MSS	F	P
Between Subjects	20	156417.1410			
C (CONC)	6	90751.8120	15125.3018	3.225	0.0331
Subj w Groups	14	65665.3280	4690.3804		

Analysis of Variance File: bromlem Date: 01-22-1991

FILTER: None

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	520.333	6	475.667
2	558.000	7	380.667
3	534.667		
4	545.000		
5	601.667		

Comparison	Tukey-A*	Newman -Keuls*	Dunnett
1 < 2			
1 < 3			
1 < 4			
1 < 5			
1 > 6			
1 > 7		0.1000	
2 > 3			N.A.
2 > 4			N.A.
2 < 5			N.A.
2 > 6			N.A.
2 > 7	0.1000	0.1000	N.A.
3 < 4			N.A.
3 > 5		0.1000	N.A.
4 < 5			N.A.
4 > 6			N.A.
4 > 7		0.1000	N.A.
5 > 6			N.A.
5 > 7	0.0500	0.0500	N.A.
6 > 7			N.A.

* The only possible P-values are .01, .05 or .10 (up to 0.1000).
A blank means the P-value is greater than 0.1000.

Page_____ is not included in this copy.

Pages 10 through 13 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

__✓__ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Shaughnessey No. 035301, 035302 Chemical Name Bromoxynil Octanoate Chemical Class ____ Page 1 of 1

Study/Species/Lab/ Accession	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
14-Day Single Dose Oral LD_{50}		LD50 = mg/kg (95% C.L.) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
14-Day Single Dose Oral LD_{50}		LD50 = mg/kg. (95% C.L.) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab		8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab		8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
~~48-Hour LC_{50}~~ 14-Day EC_{50}		EC50 = >0.25ppm* (95% C.L. N/A) Contr. Mort.(%)= N/A		
Species Lemna gibba		Slope= N/A # Animals/Level= 15 (trends) Sol. Contr. Mort.(%)= N/A		
Lab Springborn Labs. 97.2		14-Day ~~48-Hour~~ Dose Level ppm*/(%Mortality) Temperature = 19-29°C	MR 2-9-91	Inval
Acc. MRID# 416060-03		0.25 (29.3), 0.18 (11.7), 0.22 (-11.7), 0.076 (-1.1), <0.025 (0.7) Comments: Author's reported values		
96-Hour LC_{50}		LC50 = pp__ (95% C.L.) Con. Mor(%)= Sol. Con. Mor.(%)=		
Species		Slope= # Animals/Level= Temp.=		
Lab		96-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
96-Hour LC_{50}		LC50 = pp__ (95% C.L.) Con. Mort.(%)= Sol. Con. Mort.(%)=		
Species		Slope= # Animals/Level= Temp.=		
Lab		96-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		